Exhibit E

Invitrogen™
life technologies

The Most Efficient Enterokinase for Fusion Tag Cleavage

If you need to cleave your fusion protein with enterokinase, here's good news. Invitrogen's recombinant EnterokinaseMax™ (EKMax™) provides you with unsurpassed digestion efficiency. With EKMax™, you can completely cleave fusion tags using minimal amounts of enterokinase. In addition, the highly specific EK-Away™ resin effectively removes enterokinase from your digestion mixture. With EKMax™ and EK-Away™ you can obtain pure native protein for downstream experiments.

Why EKMax™?

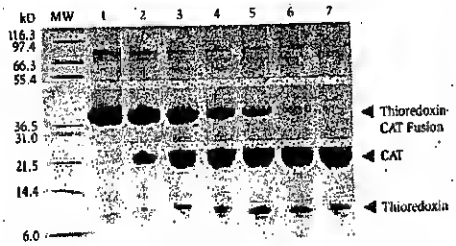
EKMax™ is a highly purified recombinant enterokinase. It recognizes the specific amino acid sequence Asp-Asp-Asp-Asp-Lys and cleaves following the lysine residue. EKMax™ is the most efficient enterokinase available, because it offers exceptional activity and specificity.

EKMax™ is the most active enterokinase around. Almost all commercially available enterokinases are porcine or bovine holoenzymes. EKMax™ is a recombinant preparation of the active catalytic subunit of bovine enterokinase (1). This means that with EKMax™ you use less enzyme than other commercial preparations and still achieve maximum digestion efficiencies.

EKMax™ is ultra specific. EKMax™ is produced in the yeast *Pichia pastoris*. This unique expression system secretes high levels of correctly processed enterokinase into the growth medium so EKMax™ contains almost no foreign proteins right from the start. This means EKMax™ has a higher specific activity and there is no unwanted cleavage caused by contaminants.

EKMax™ really works. To demonstrate how well EKMax™ works, we've used EKMax™ to digest a thioredoxin-chloramphenicol acetyl transferase (CAT) fusion protein. Figure 1 shows that protein is completely cleaved into thioredoxin and CAT with just 0.6 units* of EKMax™.

Figure 1 - Digestion of thioredoxin-CAT fusion protein with EKMax™



Each reaction contains 10 µg of partially purified thioredoxin-CAT fusion protein and varying amounts of EKMax™. Reactions were incubated at 37°C for 16 hours and analyzed on a Coomassie® blue-stained 10-20% Tricine-SDS gel. Units of EKMax™ used per reaction are listed below.

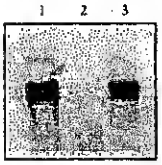
Lane 1: No EKMax™
Lane 2: 0.05 units*
Lane 3: 0.1 units
Lane 4: 0.15 units
Lane 5: 0.2 units
Lane 6: 0.4 units
Lane 7: 0.6 units

* One unit of EKMax™ is the amount of enzyme required to digest 20 µg of a thioredoxin-CAT fusion protein to 90% completion in 16 hours at 37°C. One EKMax™ unit is equivalent to ~190 trypsinogen activation units.

EK-Away™ for rapid removal of EKMax™

If removal of EKMax™ from your native protein is critical for subsequent analysis, try EK-Away™. EK-Away™ is an agarose based resin that provides a quick and efficient way to separate enterokinase (EKMax™ or enterokinase holoenzymes) from a mixture of proteins by binding to the enzyme's catalytic site. It takes only 5 minutes for the EK-Away™-enterokinase complex to form. Following brief centrifugation, greater than 99% of the enterokinase can be removed (figure 2).

Figure 2 - Western blot of EKMax™ bound to EK-Away™



One hundred and fifty microliters of EK-Away™ was added to 500µl cleavage mixture containing 5 units of EKMax™. The EK-Away™ resin from the binding reaction was spun down, suspended in Laemmli loading buffer and boiled for 1 minute to release EKMax™. Ten microliters of the boiled supernatant, along with 10 µl from the supernatant of the binding reaction, were separated by SDS-PAGE. The gel was blotted and probed using an anti-enterokinase antibody.

Lane 1: 0.1 units of EKMax™ (control)
Lane 2: 10 µl of binding reaction supernatant
Lane 3: 10 µl of supernatant from boiled resin

Quality guaranteed

To guarantee consistent quality results with EKMax™ and EK-Away, each product is stringently tested. Our quality control standards require that 1 unit of EKMax™ digests 20 µg of a thioredoxin-CAT fusion protein to 90% completion in 16 hours at 37°C. Each lot of EKMax™ is also incubated with azocasein to ensure that there is no non-specific protease activity. Each lot of EK-Away™ resin is tested by a sensitive fluorometric assay to ensure that less than 1% of enterokinase activity remains after the binding reaction.

Ordering information

EKMax™ and EK-away™ together represent the most efficient system available for enterokinase cleavage and removal. EKMax™ is supplied in 250 and 1,000 unit sizes and comes with 10X reaction buffer and a comprehensive user manual. EK-Away™ is offered in two convenient sizes that include the EK-Away™ resin, 10X binding buffer, and 10X stripping buffer. To get your ultra pure recombinant native protein, call Invitrogen and order EKMax™ and EK-Away™ today!

Description	Quantity	Cat. no.
EnterokinaseMax™ (EKMax™)	250 units	E180-01
	1,000 units	E180-02
EK-Away™	7.5 ml*	R180-01
	30 ml*	R180-02

* 7.5 ml EK-Away™ resin will remove 250 units of EKMax™; 30 ml will remove 1,000 units of EKMax™.

Reference:
1. La Vallie, E.R. *et al.* (1993) *J. Biol. Chem.* 268: 23311-23317.

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